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The combination of recombinant factor VIIa and fibrinogen correct clotting *ex vivo* in patient samples obtained following cardiopulmonary bypass surgery

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ABSTRACT

Cardiac surgery involving cardio pulmonary bypass (CPB) may be associated with development of a coagulopathy that increases risk of bleeding. In the present *ex vivo* study we investigated the influence of fibrinogen and rFVIIa, alone or in combination, on whole blood coagulation thromboelastometry using pre- and postoperative blood samples from 18 consecutive adult patients undergoing CPB surgery. Dynamic thromboelastometric clotting profiles were recorded using citrated whole blood activated with trace amounts of tissue factor (Innovin®, final dilution 1:17000). Blood samples were collected before surgery (control) and postoperative samples were obtained following *in vivo* neutralization of heparin with protamine sulphate. All blood samples were treated with heparinase to ensure neutralization of possible residual heparin effect. The post-operative blood samples were spiked with buffer, rFVIIa (2 µg/mL), fibrinogen (1 mg/mL), or the combination of rFVIIa and fibrinogen. Despite neutralization of heparin, CPB surgery left a measurable coagulopathy that was thromboelastometrically characterized by prolonged onset of clotting, reduced maximum velocity of clot formation (MaxVel), and decreased maximum clot firmness (MCF). *Ex vivo* spiking of the postoperative samples with rFVIIa shortened the clotting time. Fibrinogen also shortened the clotting time and, in addition, improved the MaxVel, and MCF. Finally, adding the combination of rFVIIa and fibrinogen to the postoperative samples corrected all thromboelastometric parameters to the preoperative range. In conclusion, the correction of whole blood clotting abnormalities that occurs with rFVIIa and/or fibrinogen suggests that future clinical trials on treatment of bleeding during CPB surgery should study the haemostatic effect of fibrinogen or possibly the combination of rFVIIa and fibrinogen.

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1. Introduction

During cardiopulmonary bypass (CPB) surgery a multifactorial coagulopathy may develop and this may contribute to peri- and post-operative bleeding. Possible causes include heparinization, surgical trauma, haemodilution, extracorporeal circulation, consumption coagulopathy, increased fibrinolysis, and hypothermia [1–3]. Despite adequate neutralization of heparin with protamine sulphate at the end of surgery [4], haemostasis often remains compromised and this may contribute to severe bleeding, leading to surgical re-exploration in 3 to 4% [4,5]. Standard medical management of the coagulopathy includes transfusion of red blood cells, fresh frozen plasma, and

platelets [6]. Clinical reports have also suggested a beneficial haemostatic effect of recombinant factor VIIa (rFVIIa) administration [7–9] or the administration of a fibrinogen concentrate or cryoprecipitate [10].

Beside whole blood rotational thromboelastometry (ROTEM®) using activation with minute amounts of tissue factor is a sensitive method that may allow tailoring of haemostatic intervention in various coagulopathies and during surgery [11–13]. The ROTEM could possibly help predict the clinical response to various haemostatic agents [14–18]. Recently, Tanaka et al reported an improvement in ROTEM parameters after addition of rFVIIa (1.5 µg/mL) and fibrinogen (1 mg/mL) alone or in combination to postoperative blood samples obtained following CPB surgery in seven patients, although no preoperative control or other details revealing a coagulopathy were shown [19].

In the current *ex vivo* study, done on whole blood samples obtained from patients before and after CPB surgery, we tested the

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ex vivo effect of rFVIIa, fibrinogen, and the combination of both on the abnormal thromboelastometric coagulation pattern that developed during surgery.

2. Materials and Methods

2.1. Study subjects

We studied eighteen consecutive adult patients undergoing cardiopulmonary bypass surgery at the Landspítali University Hospital in Reykjavik, Iceland. None of the patients were treated with aprotinin or tranexamic acid during surgery. Patients with known congenital thrombotic or haemostatic disorders were not eligible. The median age of the study population was 68 years (range 39–82). Twelve patients underwent coronary artery bypass grafting (CABG), two aortic valve replacement (AVR), three CABG + AVR, and one mitral valve repair. Nine patients had stable angina pectoris (AP) and underwent elective surgery whereas the other 9 patients presented with unstable AP and had expedited surgery. All patients with unstable AP received low molecular weight heparin (enoxaparin 80 mg) q12 hours until the day of operation. According to standard hospital procedure, patients undergoing elective surgery were recommended to discontinue aspirin prior to surgery and the aspirin was discontinued between 2 - 18 days prior to surgery (median = 8 days).

2.2. Blood sampling

Two sets of blood samples were obtained from the distal lumen (16 Ga) of the non-heparinized central venous catheter (Arrow-Howes™ Quad-Lumen, Arrow International Inc. Reading, PA, USA). The first set was obtained immediately after the patients were anaesthetized but prior to initiation of surgery and the second set 15 minutes after termination of CPB and full reversal of heparin (Leo® Pharma A/S, Copenhagen, Denmark) with protamine sulphate (Leo® Pharma A/S, Copenhagen, Denmark). The first 10 ml of aspirated blood were discarded to minimize pre-activation of the blood. For coagulation and platelet analyses we used 5 ml 3.2% sodium citrate Becton-Dickinson vacutainer® tubes (Becton Dickinson, Belliver Industrial Estate, Plymouth, United Kingdom), whereas 4 ml BD EDTA vacutainer tubes were used for measurement of the complete blood count.

2.3. Reagents and buffers

As a buffer control we used HEPES 20 mM, NaCl 150 mM, pH = 7.4 (Bie & Berntsen A-S Herlev, Denmark). Recombinant factor VIIa (rFVIIa, NovoSeven®, Novo Nordisk, Bagsvaerd, Denmark) and fibrinogen concentrate (Haemocomplettan®, CSL Behring, Marburg, Austria) were obtained from the manufacturer. The HEPTEM® reagent from Pentapharm, Munich, Germany was used as source of heparinase. The tissue factor (TF) source was Innovin® from Dade Behring, Marburg, Germany.

2.4. Whole blood coagulation analyses

Dynamic whole blood clot formation profiles were recorded by a ROTEM® Thromboelastometry Coagulation Analyzer (Pentapharm, München, Germany). The analytical methodology adopted is described elsewhere [20]. In brief, citrated blood samples rested for 30 minutes at ambient temperature. The reaction mixture contained 280 µl of citrated whole blood + 10 µl of heparinase + 20 µl buffer or drug (final added concentrations: rFVIIa 2 µg/mL corresponding to a 90 µg/kg dose and/or fibrinogen 1 mg/mL corresponding to a 3 g dose in a 80 kg person) + 20 µl TF and CaCl₂ 200 mM. All results shown are based on means of duplicate experiments. The thromboelastometry measurement of whole blood clot formation was based on activation with minimal amounts of tissue factor (final reaction mixture dilution

1:17,000). Assessment of whole blood clot formation was based on standard thromboelastometry parameters such as the clotting time (CT) and the maximum clot firmness (MCF). In addition, the ROTEM® raw data were processed using the DyCoDerivAn™ software (Avordusol, Risskov, Denmark) providing dynamic velocity profiles and derived parameters such as the maximum velocity (MaxVel) and time to maximum velocity (t, MaxVel) of clot formation [20]. The CT characterizes the initiation phase of whole blood clot formation. The MaxVel and t, MaxVel, define the propagation phase of whole blood clotting. The stabilization phase is expressed by the MCF and is predominantly sensitive to platelet count and level of fibrinogen [21].

2.5. Whole blood platelet function analyses

Whole blood platelet function was evaluated by detection of PFA-100 closure times using the Platelet Function Analyser PFA-100® (Dade-Behring, Marburg, Germany) as well as Dade® PFA collagen/epinephrine (CT c/epi) and Dade® PFA Collagen/ADP (CT c/ADP) test cartridges. In brief, 800 µL of citrated whole blood were pipetted into the test cartridge. By vacuum pressure, the whole blood flows through a micro-aperture in the cartridge membrane that is coated with collagen and either ADP or epinephrine. The instrument monitors reductions in the blood flow rate as the platelets form a haemostatic obstruction in the aperture. Arrest of blood flow is denoted as closure time and the maximal value obtainable is 300 s.

2.6. Other laboratory coagulation analyses

The STA-R coagulation analyzer (Diagnostica Stago, Asnieres, France) was used for the following plasma coagulation, chromogenic tests and immunoturbidometric tests. The activated partial thromboplastin time (APTT, sec), prothrombin time (PT, sec), fibrinogen concentration (g/L), thrombin time (TT, sec) were measured using Platelin® LS (BioMérieux USA), Tissue factor STA® Néoplastine® CI plus, STA®-FIBRINOGEN 5, and STA®-Thrombin, respectively. The activity of antithrombin, protein C, and antiplasmin was measured using STA®-STACHROM® AT III, STA®-STACHROM® PROTEIN C, and STA®-STACHROM® ANTIPLASMIN, respectively. D-dimer was determined using the STA®-LIATEST®D-DI reagent. The reagents were obtained from Diagnostica Stago unless stated otherwise.

2.7. Statistical considerations

The distribution of data was evaluated using histograms and Q-Q-plots. Data did not follow a Gaussian distribution, hence data comparison was performed using the non parametric Wilcoxon signed rank test for paired data. Results are shown as median (range). Statistical significance was defined by a p-value < 0.05.

2.8. Ethical considerations

The study was approved by the Ethical Committee of the Landspítali University Hospital in Reykjavik, Iceland and the Data Protection Authority of Iceland. Informed consent was obtained from all participants prior to surgery.

3. Results

3.1. Clinical data

The median time on cardiopulmonary bypass was 102 minutes (range 45–206). The median cross clamp time was 54 minutes (range 21–153). The median lowest core body temperature was 35 °C (33.2–35.7). The median blood loss during the operation measured based on suctioned waste and weight of used gauzes and sheets was 1055 mL (10 – 3750). In the 18 patients, there was a significant positive correlation between the

Table 1
Pre- and postoperative standard coagulation tests and blood cell count.

| | Pre-operative values | Post-operative values |
|--|----------------------|-----------------------|
| | Median (range) | Median (range) |
| APTT [sec] | 36.7 (29.7-48.5) | 47.0 (35.9-60)*** |
| Thrombin time [sec] | 16.6 (15.4-17.6) | 17.0 (14.7-19.6)* |
| PT [sec] | 14.2 (13.2-15.7) | 18.4 (17.1-22.9)*** |
| Fibrinogen [g/L] | 3.1 (2-5.4) | 2.0 (1.5-2.7)* |
| D-Dimer [mg/L] | 0.33 (0.24-8.2) | 3.3 (1.5-16.3)*** |
| Antithrombin [U/dL] | 85 (69-108) | 64 (49-80)*** |
| Antiplasmin [U/dL] | 84 (65-105) | 58 (40-73)*** |
| Protein C [U/dL] | 106 (91-166) | 82 (64-115)*** |
| White blood cells ($\times 10^9/L$) | 5.4 (2.7-9.5) | 11.7(3.4-19.2)*** |
| Red blood cells ($\times 10^{12}/L$) | 4.1 (2.9-4.8) | 3.2(2.29-3.86)*** |
| Haemoglobin (g/L) | 126 (123-129) | 100 (82-116)*** |
| Haematocrit (%) | 36 (26-42) | 29 (26-42)*** |
| Platelets ($\times 10^9/L$) | 222 (165-406) | 123(72-237)*** |

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Wilcoxon signed rank test.

Lists standard coagulation tests as well as the blood cell count before and immediately after cardiac surgery.

reduction in measured fibrinogen concentration (g/L) during surgery and the amount of intra-operative blood loss (R^2 0.37, $p = 0.0069$, data not shown). The use of the heart and lung machine resulted in a positive fluid balance of 2820 (1920-4530). The median postoperative blood loss for 24 hours following chest closure assessed based on chest tube drainage was 900 mL (300-3600).

3.2. Standard coagulation parameters and blood cell count

As evident from Table 1, a moderate coagulopathy as well as hyperfibrinolysis was present following CPB based on measured

changes in APTT, PT, TT, Clauss fibrinogen, protein C, antithrombin, antiplasmin, and D-dimer. Moreover, the white blood cell count increased and the red blood cells, haemoglobin, haematocrit, and platelets decreased.

3.3. Whole blood thromboelastometric profiles following cardiac surgery with and without spiking with fibrinogen and recombinant factor VIIa

Despite neutralization of heparin with protamine and heparinase, the dynamic whole blood coagulation profiles were significantly abnormal following CPB. Illustrative results from a single patient are shown in Fig. 1, panel A. There is a prolonged initiation phase (prolonged CT), a compromised propagation phase as measured by a lower MaxVel and a longer t , MaxVel, and a reduced whole blood clot firmness (a diminished MCF). All ROTEM results (CT, MaxVel, t , MaxVel, and MCF) are shown in Table 2. *Ex vivo* spiking experiments with fibrinogen and rFVIIa, separately or together, on blood samples taken following ACT-guided reversal of heparin with protamine and after mixing with heparinase showed that both haemostatic agents improved the abnormal ROTEM parameters (Table 2). Thus, *ex vivo* spiking with rFVIIa significantly shortened the CT and the t , MaxVel, but rFVIIa did not induce significant changes in the MaxVel or MCF. With the addition of fibrinogen a significant shortening of the CT and t , MaxVel and increased MCF occurred as well as a borderline improvement in the MaxVel ($p = 0.05$). The addition of fibrinogen and rFVIIa together revealed further improvement, i.e. a full correction of all the coagulopathic ROTEM findings to pre-treatment levels as illustrated by a shortening of the CT and t , MaxVel and an increase in the MaxVel and normalization of the MCF.

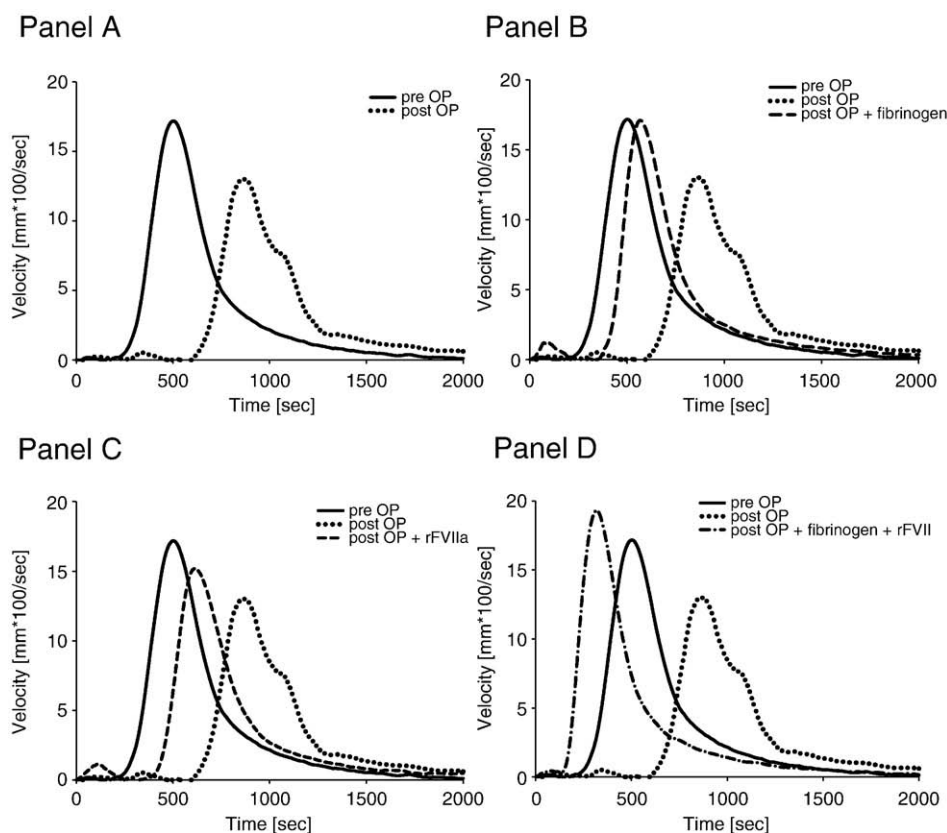


Fig. 1. Whole blood dynamic thromboelastometry profiles before and after cardiac surgery – effect of fibrinogen and recombinant factor VIIa. Panel A: Illustrates whole blood clotting velocity profiles before and after cardiac surgery following neutralization of heparin with protamine in vivo and heparinase *ex vivo*. Panels B, C, and D: Visualizes the haemostatic effect of *in vitro* addition of fibrinogen (B), recombinant factor VIIa (C) as well as the combination of fibrinogen and recombinant factor VIIa (D) to the postoperative sample.

Table 2
Whole blood thrombelastometry parameters – effect of fibrinogen, recombinant factor VIIa and the combination of fibrinogen and recombinant factor VIIa.

| | Pre-operative values | Post-operative values | Post-operative values + rFVIIa | Post-operative values + fibrinogen | Post-operative Values + rFVIIa & fibrinogen |
|-----------------------|----------------------|-----------------------|--------------------------------|------------------------------------|---|
| | Median (range) | Median (range) | Median (range) | Median (range) | Median (range) |
| Clot initiation | | | | | |
| - CT (sec) | 183 (147-475) | 385 (375-558)** | 232 (62-702)▯▯ | 246 (45-696)▯ | 155 (55-601)▯▯▯ |
| Clot propagation | | | | | |
| - MaxVel (mm*100/sec) | 17.5 (7.3-28.7) | 15.1 (8.1-21.2)** | 15.2 (8.5-22.4) | 16.0 (11.3-24.0) | 16.8 (10.2-25.7)▯ |
| - t, MaxVel (sec) | 368 (282-780) | 560 (556-766)* | 436 (188-926)▯ | 409 (194-1002)▯ | 312 (200-804)▯▯ |
| Clot stabilization | | | | | |
| - MCF (mm) | 6234 (4965-7939) | 5527 (4549-6736)**** | 5501 (3685-6762) | 5839 (5154-6889)▯▯▯ | 5808 (5100-8163)▯▯ |

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to pre-operative value by Wilcoxon signed rank test.

▯p<0.05, ▯▯p<0.01, ▯▯▯p<0.001 compared to post-operative value by Wilcoxon signed rank test.

The table shows the numerical results of the whole blood thrombelastometry parameters before and after cardiac surgery as well as following addition of the haemostatic components fibrinogen and recombinant factor VIIa. The thrombelastometry clotting process is described by an initiation phase followed by a propagation phase and a stabilization phase.

3.4. Whole blood platelet function

Prior to surgery, eight patients had abnormal PFA-100 closure times; seven had prolonged CT c/epi and 7 patients prolonged CT c/ADP. All five patients undergoing aortic valve replacement had prolonged CT c/ADP and 4 out of 5 had prolonged CT c/epi pre-operatively. Surgery induced a significant further prolongation of the CT c/epi assay ($p<0.02$, Fig. 2, panel A) but not of the CT c/ADP ($p = n.s.$, Fig. 2, panel B).

4. Discussion

Based on comparison of ROTEM patterns recorded in pre- and postoperative blood samples obtained from CPB-surgical patients, we found that *ex vivo* addition of the combination of rFVIIa and fibrinogen in pharmacological concentrations corrected the abnormal postoperative thrombelastometric abnormalities into the preoperative range. We also confirmed the previously published findings of Tanaka et al showing that rFVIIa or fibrinogen as single agents *ex vivo* partially corrected the postoperative ROTEM abnormalities in whole blood samples obtained from CPB patients after reversal of heparin with protamine sulphate [19]. In that paper further correction was observed by applying both agents together but since no preoperative control samples were investigated the full degree of correction could not be shown. Interestingly, both studies show that the *ex vivo* correction occurs irrespective of the heterogenous nature of the postoperative coagulopathy which involves haemodilution, loss or consumption of

many coagulation factors, increased fibrinolysis and loss of platelets or platelet function. Due to our use of heparinase, the coagulopathy present in our study is less likely to be caused by a residual heparin effect. Therefore, our findings are unlikely caused by possible reversal of a low dose heparin effect as has been shown to occur with rFVIIa in a rat model [22].

The findings that we and Tanaka et al describe are to our knowledge the first studies that demonstrate an additive effect of rFVIIa and fibrinogen on coagulopathic samples obtained from CPB-patients. The corrective effect of fibrinogen alone is also notable. Similar findings were previously shown in *in vitro* studies based on *in vitro* albumin-buffer dilutions of normal plasma [23] and in crystalloid induced dilutional coagulopathy [24]. Interestingly, a recent clinical study in CPB-patients showed that postoperative bleeding correlated with the preoperative fibrinogen concentration [25] and in our study intraoperative bleeding in the 18 patients correlated with fibrinogen loss, both supporting the increasingly evident importance of the concentration of fibrinogen [26]. Also, noteworthy, a recent prospective randomized study has shown that in patients undergoing radical cystectomy, fibrinogen supplementation improve the ROTEM findings as well as reduce the requirement of postoperative blood transfusion [27].

Recombinant factor VIIa in a pharmacological concentration facilitates thrombin generation by activating factor X on the surface of activated platelets by both tissue factor dependent [28] and tissue factor independent mechanisms [29,30]. In the present experiments, rFVIIa alone primarily shortened the initiation phase (CT) of whole blood clot formation, whereas the haemostatic effect on the compromised

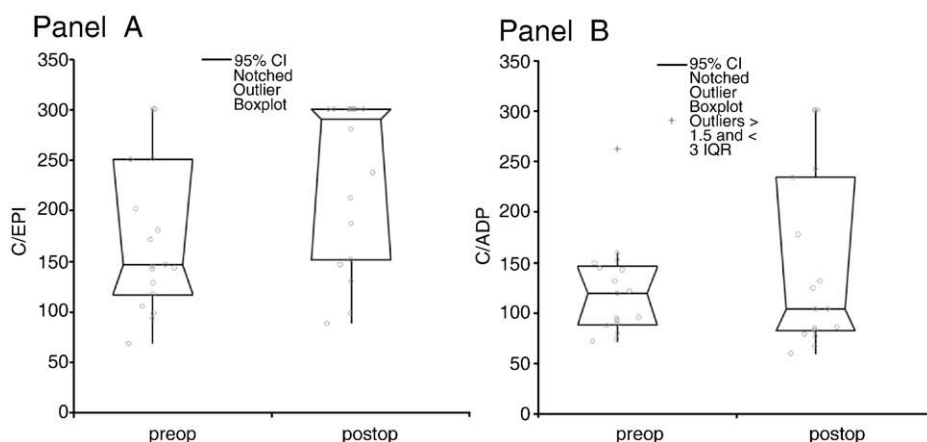


Fig. 2. Platelet function before and after cardiac surgery. Platelet function evaluated by PFA-100. The figures illustrate the closure time (sec) before and after cardiac surgery using collagen and epinephrine cartridge (c/epi, Panel A) or the collagen and ADP cartridge (c/ADP, Panel B). The median values of the c/epi measurements are significantly prolonged post-operatively ($p<0.02$) but the c/ADP do not differ.

propagation phase (MaxVel) and stabilization phase (MCF) was insignificant. The insignificant effect of rFVIIa alone on clot propagation and stabilization could possibly be explained by the reduction in postoperative platelet number and function or, alternatively, by a reduced concentration of substrate for the rFVIIa facilitated thrombin generation, i.e. hypo- or dysfibrinogenemia caused by consumption, haemodilution coagulopathy, or increased fibrinolysis. The beneficial effect of fibrinogen supplementation *ex vivo* in our experiments could therefore possibly be due to reversal of an acquired hypofibrinogenemia or dysfunction of fibrin polymerization. The latter has been shown to develop secondary to infusion of colloid plasma expanders or gelatins which are commonly used during CPB surgery [11,31]. At the single added pharmacological concentrations of rFVIIa and fibrinogen applied in our experiments, fibrinogen alone showed a stronger pro-haemostatic effect *ex vivo* than rFVIIa alone, i.e. the initiation, propagation ($p = .05$), and stabilization phases were all improved with fibrinogen. A speculative, but plausible explanation of the additive effect found after spiking with both rFVIIa and fibrinogen on ROTEM parameters, may be related to the effect of accelerated thrombin generation induced by rFVIIa which in the presence of added fibrinogen may enhance the stability of the fibrin network generated [32].

Noteworthy, levels of fibrinogen are most often measured using the Clauss method. Unfortunately, this method may reveal false high levels of fibrinogen due to e.g. haemodilution and the presence of colloid plasma expanders. In contrast, global haemostatic assays like thromboelastometry clearly illustrate abnormal fibrin polymerization and fibrinogen deficiency.

The current study was an experimental *ex vivo* study that used whole blood thromboelastometry as an endpoint. Since our study involved only 18 patients with heterogeneous heart conditions, no clinical correlation of the *ex vivo* effect of rFVIIa and fibrinogen was attempted. Optimally, however, clinical outcome data, such as the incidence of re-operation due to bleeding or the measured blood loss, would be preferable. Therefore, it may only be speculated if our results indicate a beneficial clinical effect on hemostasis in patients. However, a recent study found that abnormal thromboelastometry correctly identified hypocoagulable dogs with bleeding with a positive predictive value of 89% and a negative predictive value of 98% [33] and, therefore, it seems possible that the improved ROTEM pattern indicates improved hemostasis, although further clinical correlations are needed. Based on this it may be speculated that during treatment of intractable bleeding following CPB surgery early fibrinogen supplementation should be considered, either as the first choice or in combination with rFVIIa. However, we emphasize that clinical safety and dose ranging studies are needed before any clinical application of our findings can be recommended.

Our study has further limitations. None of our patients received anti-fibrinolytics such as tranexamic acid or aprotinin which are commonly used during CPB [34] and these agents could have improved the thromboelastometric profiles as shown in other circumstances [18]. Also, all five patients undergoing aortic valve replacement had abnormal pre-operative platelet function as measured by the CT c/ADP. The CT c/ADP prolongation is most commonly found in patients with low von Willebrand factor (VWF) [35]. Although VWF was not measured, this phenomenon could be a sign of acquired breakdown of VWF (acquired von Willebrand disease) in consequence of the aortic valve disease (equivalent to von Willebrand disease type 2A) as previously suggested by others [36].

We conclude that both rFVIIa and fibrinogen have a positive effect on CPB-associated thromboelastometric abnormalities *ex vivo* and that their combined supplementation normalizes the abnormal thromboelastogram to pre-treatment values. Currently, clinical studies are ongoing that use rFVIIa to control hemorrhage during cardiac surgery. Our data suggests that future clinical trials in cardiac surgery should also study the haemostatic effect and safety of fibrinogen and possibly the combination of rFVIIa and fibrinogen.

References

- [1] Linden MD. The hemostatic defect of cardiopulmonary bypass. *J Thromb Thrombolysis* 2003;16:129–47.
- [2] Hartmann M, Sucker C, Boehm O, Koch A, Loer S, Zacharowski K. Effects of cardiac surgery on hemostasis. *Transfus Med Rev* 2006;20:230–41.
- [3] Despotis GJ, Avidan MS, Hogue Jr CW. Mechanisms and attenuation of hemostatic activation during extracorporeal circulation. *Ann Thorac Surg* 2001;72:S1821–31.
- [4] Karthik S, Grayson AD, McCarron EE, Pullan DM, Desmond MJ. Reexploration for bleeding after coronary artery bypass surgery: risk factors, outcomes, and the effect of time delay. *Ann Thorac Surg* 2004;78:527–34.
- [5] Dacey LJ, Munoz JJ, Baribeau YR, Johnson ER, Lahey SJ, Leavitt BJ, et al. Reexploration for hemorrhage following coronary artery bypass grafting: incidence and risk factors. *Northern New England Cardiovascular Disease Study Group. Arch Surg* 1998;133:442–7.
- [6] Ferraris VA, Ferraris SP, Saha SP, Hessel EA, Haan CK, Royston BD, et al. Perioperative blood transfusion and blood conservation in cardiac surgery: the Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists clinical practice guideline. *Ann Thorac Surg* 2007;83:S27–86.
- [7] Al Douri M, Shafi T, Al Khudairi D, Al Bokhari E, Black L, Akinwale N, et al. Effect of the administration of recombinant activated factor VII (rFVIIa; NovoSeven) in the management of severe uncontrolled bleeding in patients undergoing heart valve replacement surgery. *Blood Coagul Fibrinolysis* 2000;11(Suppl 1):S121–7.
- [8] Brandsborg S, Sørensen B, Poulsen LH, Ingerslev J. Recombinant activated factor VIIa in uncontrolled bleeding: a haemostasis laboratory study in non-haemophilia patients. *Blood Coagul Fibrinolysis* 2006;17:241–9.
- [9] Karkouti K, Beattie WS, Wijeyesundera DN, Yau TM, McCluskey SA, Ghannam M, et al. Recombinant factor VIIa for intractable blood loss after cardiac surgery: a propensity score-matched case-control analysis. *Transfusion* 2005;45:26–34.
- [10] Rossaint R, Duranteau J, Stahel PF, Spahn DR. Nonsurgical treatment of major bleeding. *Anesthesiol Clin* 2007;25:35–48 viii.
- [11] Fenger-Eriksen C, Anker-Møller E, Heslop J, Ingerslev J, Sørensen B. Thromboelastographic whole blood clot formation after *ex vivo* addition of plasma substitutes: improvements of the induced coagulopathy with fibrinogen concentrate. *Br J Anaesth* 2005;94:324–9.
- [12] Sørensen B, Ingerslev J. Tailoring haemostatic treatment to patient requirements - an update on monitoring haemostatic response using thromboelastography. *Haemophilia* 2005;11(Suppl 1):1–6.
- [13] Sørensen B, Ingerslev J. A direct thrombin inhibitor studied by dynamic whole blood clot formation. Haemostatic response to *ex vivo* addition of recombinant factor VIIa or activated prothrombin complex concentrate. *Thromb Haemost* 2006;96: 446–53.
- [14] Sørensen B, Ingerslev J. Whole blood clot formation phenotypes in hemophilia A and rare coagulation disorders. Patterns of response to recombinant factor VIIa. *J Thromb Haemost* 2004;2:102–10.
- [15] Sørensen B, Persson E, Ingerslev J. Factor VIIa analogue (V158D/E296 V/M298Q-FVIIa) normalises clot formation in whole blood from patients with severe haemophilia A. *Br J Haematol* 2007;137:158–65.
- [16] Larsen OH, Clausen N, Persson E, Ezban M, Ingerslev J, Sørensen B. Whole blood coagulation in children with thrombocytopenia and the response to platelet replacement, recombinant factor VIIa, and a potent factor VIIa analogue. *Br J Haematol* 2008;144(1): 99–106.
- [17] Ingerslev J, Poulsen LH, Sørensen B. Potential role of the dynamic properties of whole blood coagulation in assessment of dosage requirements in haemophilia. *Haemophilia* 2003;9:348–52.
- [18] Hvas AM, Sørensen HT, Norengaard L, Christiansen K, Ingerslev J, Sørensen B. Tranexamic acid combined with recombinant factor VIII increases clot resistance to accelerated fibrinolysis in severe hemophilia A. *J Thromb Haemost* 2007;5:2408–14.
- [19] Tanaka KA, Taketomi T, Szlam F, Calatzis A, Levy JH. Improved clot formation by combined administration of activated factor VII (NovoSeven) and fibrinogen (Haemocomplettan P). *Anesth Analg* 2008;106:732–8 table.
- [20] Sørensen B, Johansen P, Christiansen K, Wöelke M, Ingerslev J. Whole blood coagulation thromboelastographic profiles employing minimal tissue factor activation. *J Thromb Haemost* 2003;1:551–8.
- [21] Lang T, Johanning K, Metzler H, Piepenbrock S, Solomon C, Rahe-Meyer N, et al. The effects of fibrinogen levels on thromboelastometric variables in the presence of thrombocytopenia. *Anesth Analg* 2009;108:751–8.
- [22] Lauritzen B, Hedner U, Johansen PB, Tranholm M, Ezban M. Recombinant human factor VIIa and a factor VIIa-analogue reduces heparin and low molecular weight heparin (LMWH)-induced bleeding in rats. *J Thromb Haemost* 2008;6:804–11.
- [23] Ganter MT, Schmuck S, Hamiel CR, Wischmeyer PE, Heule D, Zollinger A, et al. Monitoring recombinant factor VIIa treatment: efficacy depends on high levels of fibrinogen in a model of severe dilutional coagulopathy. *J Cardiothorac Vasc Anesth* 2008;22:675–80.
- [24] Fenger-Eriksen C, Tonnesen E, Ingerslev J, Sørensen B. Recombinant factor VIIa and fibrinogen display additive effect during *in vitro* haemodilution with crystalloids. *Acta Anaesthesiol Scand* 2009;53:332–8.
- [25] Karlsson M, Ternstrom L, Hyllner M, Baghaei F, Nilsson S, Jeppsson A. Plasma fibrinogen level, bleeding, and transfusion after on-pump coronary artery bypass grafting surgery: a prospective observational study. *Transfusion* 2008;48:2152–8.
- [26] Nielsen VG, Levy JH. Fibrinogen and bleeding: old molecule–new ideas. *Anesth Analg* 2007;105:902–3.
- [27] Fenger-Eriksen C, Jensen TM, Kristensen BS, Jensen KM, Tonnesen E, Ingerslev J, et al. Fibrinogen substitution improves whole blood clot firmness after dilution with hydroxyethyl starch in bleeding patients undergoing radical cystectomy: a randomized, placebo-controlled clinical trial. *J Thromb Haemost* 2009;7:795–802.

- [28] Butenas S, Brummel KE, Bouchard BA, Mann KG. How factor VIIa works in hemophilia. *J Thromb Haemost* 2003;1:1158–60.
- [29] Hoffman M, Monroe III DM. The action of high-dose factor VIIa (FVIIa) in a cell-based model of hemostasis. *Semin Hematol* 2001;38:6–9.
- [30] Persson E, Kjalke M, Olsen OH. Rational design of coagulation factor VIIa variants with substantially increased intrinsic activity. *Proc Natl Acad Sci U S A* 2001;98:13583–8.
- [31] Fries D, Krismer A, Klingler A, Streif W, Klima G, Wenzel V, et al. Effect of fibrinogen on reversal of dilutional coagulopathy: a porcine model. *Br J Anaesth* 2005;95:172–7.
- [32] Wolberg AS. Thrombin generation and fibrin clot structure. *Blood Rev* 2007;21:131–42.
- [33] Wiinberg B, Jensen AL, Rozanski E, Johansson PI, Kjølgaard-Hansen M, Tranholm M, et al. Tissue factor activated thromboelastography correlates to clinical signs of bleeding in dogs. *Vet J* 2007;179(1):121–9.
- [34] Sedrakyan A, Treasure T, Elefteriades JA. Effect of aprotinin on clinical outcomes in coronary artery bypass graft surgery: a systematic review and meta-analysis of randomized clinical trials. *J Thorac Cardiovasc Surg* 2004;128:442–8.
- [35] Podda GM, Bucciarelli P, Lussana F, Lecchi A, Cattaneo M. Usefulness of PFA-100 testing in the diagnostic screening of patients with suspected abnormalities of hemostasis: comparison with the bleeding time. *J Thromb Haemost* 2007;5:2393–8.
- [36] Vincentelli A, Susen S, Le TT, Six I, Fabre O, Juthier F, et al. Acquired von Willebrand syndrome in aortic stenosis. *N Engl J Med* 2003;349:343–9.